

Protein Design is a Key Factor for Subunit-subunit Association

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Abstract

Fundamental questions about role of the quaternary structures are addressed using a statistical mechanics off-lattice model of a dimer protein. The model, in spite of its simplicity, captures key features of the monomer-monomer interactions revealed by atomic force experiments. Force curves during association and dissociation are characterized by sudden jumps followed by smooth behavior and form hysteresis loops. Furthermore, the process is reversible in a finite range of temperature stabilizing the dimer. It is shown that in the interface between the two monomeric subunits the design procedure naturally favors those amino acids whose mutual interaction is stronger. Furthermore it is shown that the width of the hysteresis loop increases as the design procedure improves, i.e. stabilizes more the dimer.

I. INTRODUCTION

Molecular recognition is a process by which two biological molecules interact to form a specific complex. Structural domains of proteins recognize ligands, nucleic acid and other proteins in nearly all fundamental biological processes. The recognition comprises a large spectrum of specific non bonded interactions, such as van der Waals interactions, hydrogen bonding and salt bridges, which overcame the loss of conformational entropy upon association [1]. These interactions are ubiquitous, yet they are responsible for the exquisite specificity of the aggregation. Understanding the aggregation process is important not only for our comprehension of the formation of the molecular aggregates but also to gain insights on how the interactions cancel each other in the many other possible supramolecular modes of association. Investigating the nature of association can also shed light on the protein folding processes, as the aggregation process can be described as the transfer of surface from water to the protein interior. Useful information could also be obtained on the complex relationship between cooperativity and quaternary structure in proteins such as myoglobin. Finally, such type of studies can have important application in pharmacology and medicine. A typical example in this respect is insulin, a protein whose efficiency for the treatment of insulin-dependent diabetes could be boosted by a better understanding of the association/dissociation mechanism [2]. A deep understanding of the aggregation mechanism is of current interest also in anti-AIDS research. Subunit-subunit association inhibitors of the dimeric enzyme HIV-1 protease are currently putative agents against HIV infection [3]. These drugs are ligands with high affinity at the interface region.

While several biochemical and biophysical experiments have been directed toward characterizing thermodynamic [1,4] and kinetic [5] aspects of protein-protein interactions, a small number of experimental [6] and theoretical [7] studies have addressed the role of subunit association at the atomic level.

In this respect, an elegant experiment is represented by the determination of the monomeric structure of a dimeric enzyme, Cu, Zn- superoxide dismutase obtained by mutat-

ing key residues at the subunit-subunit interface [6]. The three dimensional structure of the single subunit exhibits small differences with the native enzyme, yet the conformation is less favorable of the substrate to the reaction site, indicating an essential role of the quaternary structure.

Subunit-subunit interactions have been recently measured by direct force measurements. Yip et al. [8] have revealed the complexity of the insulin dimer dissociation and have shown the energetics associated to the disruption of discrete molecular bonds at the monomer-monomer interface. While these studies are capable to quantify the forces governing protein aggregation, the fundamental question on how the *design* quality of proteins (namely its topology and its three dimensional structure) affects domains of different subunits has never been addressed. Here, we use a simple statistical mechanical model to investigate the relationship between the design of a dimeric protein and the interactions present the subunit-subunit interface. Comparison is made with experiments such as those of Yip et al. [8] by calculating the forces necessary to pull away and push back the two subunits. We find that our model not only is able to capture key aspects of these experiments, but also provides novel information on the intricate relationship between the design of a dimeric protein and the interactions present the subunit-subunit interface.

II. COMPUTATIONAL SECTION

Energetics [9]. We use a very simple protein model in which only the C_α atoms are considered [11], and a very simple form of the monomer-monomer interaction energy:

$$V = V^A + V^B + V^{AB}, \quad (1)$$

where V^A (V^B) is the potential energy of N_A (N_B) interacting beads constituting chain A (B):

$$V^A = \sum_{i < j} \sum_{i=1, N_A} \left\{ \delta_{i,j+1} f(r_{i,j}) + \eta(a_i, a_j) \left[\left(\frac{\sigma}{r_{ij}^A} \right)^{12} - \left(\frac{\sigma}{r_{ij}^A} \right)^6 \right] \right\}, \quad (2)$$

and V^{AB} is the potential energy given by the interaction of beads of chain A with beads of chain B :

$$V^{AB} = \sum_{i=1, \dots, N_A} \sum_{j=1, \dots, N_B} \left\{ \eta(a_i, a_j) \left[\left(\frac{\sigma}{r_{ij}^{AB}} \right)^{12} - \left(\frac{\sigma}{r_{ij}^{AB}} \right)^6 \right] \right\}. \quad (3)$$

In eq. (2) $r_{ij}^A = |\mathbf{r}_i^A - \mathbf{r}_j^A|$ represents the distance between beads i and j of chain A, whereas in eq. (3) $r_{ij}^{AB} = |\mathbf{r}_i^A - \mathbf{r}_j^B|$ is the distance between bead i of chain A and bead j of chain B. The parameters $\eta(a_i, a_j)$ and σ entering these equations determine, respectively, the energy scale and the interaction range between monomer kinds a_i and a_j . Twenty different beads are used to represent the natural amino acids present in proteins. Interaction parameters between each amino acid pair $i, j = 1, \dots, 20$ are chosen to be all attractive (i.e. $\eta(a_i, a_j) > 0 \forall (i, j)$) and proportional to the values determined by Miyazawa and Jernigan (MJ) [12]. The function $f(x)$ in eq. (2) represents the energy of the virtual C_α - C_α peptide bond and it is equal to:

$$f(x) = a(x - d_0)^2 + b(x - d_0)^4, \quad (4)$$

with a and b taken to be 1 and 100 respectively, and d_0 set equal to 3.8 Å. The effect of $f(x)$ is to act as a “soft clamp” to keep subsequent residues at nearly the typical distance observed in real proteins.

Protein model. We construct a model of a dimeric protein with a given symmetry \mathcal{S} by selecting a compact, low-energy configuration. To build the 3- D structure we follow the procedure of ref. [11]. An homopolymer is collapsed through molecular dynamics with a potential in Eq.(1) where all the η 's are taken equal to their maximum (i.e. the most attractive) value $\eta_{max} = 10$, and σ is chosen to be equal to $\sigma_0 = 6.5\text{\AA}$. In practice we consider the motion of chain A only under the potential:

$$V = V^A + \frac{1}{2} V^{A \mathcal{S}(A)}, \quad (5)$$

where $\mathcal{S}(A)$ is the chain configuration obtained from of the application of the symmetry transformation \mathcal{S} to the chain A . The potential $V^{A \mathcal{S}(A)}$ depends only on the coordinates of

chain A . In the case of a C_2 symmetry, if (x_i, y_i, z_i) $i = 1, \dots, N_A$ are the coordinates of beads of chain A , then $(-x_i, -y_i, z_i)$ $i = 1, \dots, N_A$ are the coordinates of those in chain $\mathcal{S}(A)$ and the expression of $V^{AS(A)}$ is:

$$V^{AS(A)} = \sum_{i,j=1,\dots,N_A} \left\{ \eta \left(\left(\frac{\sigma_0}{\tilde{r}_{ij}} \right)^{12} - \left(\frac{\sigma_0}{\tilde{r}_{ij}} \right)^6 \right) \right\} \quad (6)$$

where $\tilde{r}_{ij} = \sqrt{(x_i + x_j)^2 + (y_i + y_j)^2 + (z_i - z_j)^2}$.

The target conformation of the dimer ("native state") is obtained by collapsing a randomly generated swollen configuration of a chain made up of 50 monomers by using Molecular Dynamics (MD) simulations combined with a slow cooling procedure. The procedure is repeated several times from different random initial conditions (for details see [11]).

The procedure of ref. [11] is used to assign a suitable sequence to the selected structure. We used twenty kinds of amino acids a_i $i = 1, \dots, 20$, with the values of the matrix elements $\eta(a_i, a_j)$ $i, j = 1, \dots, 20$ as given in ref. [12]. The compositions of both the sequences (of chain A and B) –shown in Table I are chosen as the ones occurring in real proteins [13].

Following ref. [11] small variations in the Lennard–Jones length parameter are allowed. Here 6 possible values ($\sigma_0 = 6.5$, $\sigma_1 = 6$, $\sigma_2 = 6.25$, $\sigma_3 = 7.0$, $\sigma_4 = 7.5$ and $\sigma_5 = 8.0$ Å) are permitted, both for potential V^A (V^B) and V^{AB} . The three-dimensional structure and the intra- and inter- chain contacts as obtained from our design procedure are shown in Fig. 1. Forces as a function of the distance r of the centers of mass of the two subunits are calculated *i)* by positioning two monomers at the distance r with the conformation of the native protein ("static force"); *ii)* by performing constrained Molecular Dynamics (CMD) simulations [14] [15] [16], in order to equilibrate the system with r as constraint coordinate.

III. RESULTS

Energy landscape and folding. Almost all dimeric globular proteins exhibit symmetric or pseudo symmetric aggregation. Due to the amino acid chirality, only symmetry point groups not containing the inversion are possible. By far the most common point group

symmetry is the $C2$. Examples range from HIV-1 protease to Cu,Zn-superoxide dismutase, immunoglobulin and glutathione reductase and many others [1]. We therefore impose a $C2$ symmetry to the native state of the model dimeric protein. The target structure ("native state") is chosen as the lowest-energy conformer for the two subunits equipped with the designed sequences, A and B (see Computational Section).

To explore the energy landscape of the dimeric protein, we first collected several configurations of the subunits during MD simulations.

Fig. 2 shows the energy of each configuration of the two chains A and B plotted against the distance, d , from the dimer native state. The distance, among configurations is measured by using the Kabsch expression [17].

The two subunits are able to find the lowest minimum in a reasonable time (e.g. in a time observable by a MD simulation – typical simulation runs are about 10.000τ) only if starting from a set of initial conditions. Obviously, if all beads of chain A are initially located too far –with respect to the Lennard–Jones interacting distances σ – from any bead of chain B , the two chains would evolve independently, and the dimer structure could not be reached.

Subunit-subunit forces. These interactions are experimentally measured by attaching part of the subunits to opposing surfaces and measuring the resulting forces at several subunit-subunit distances [8]. To mimic the anchoring of the subunits, we carry out MD simulations in which the distance r between the centers of mass of the subunits is constrained.

Fig. 3 plots the constrained force as a function of r at a fixed temperature ($=0.06 \varepsilon$) [9]. Both the force required to dissociate the dimer and that to reform it are reported. Several interesting features emerge from this graph. First, both association and dissociation forces experience sudden jumps followed by a smooth behavior of the force [8,26–30]. Second, by pulling and pushing back the subunits, an hysteresis-like circle is formed. Both behaviors are observed also in experiments of protein dimers [8,26–30]. Consistently, a careful analysis of the contacts between the aminoacids of the different subunits indicates that the number of contacts destroyed during dissociation at a given intermonomer distance r is *not* equal to

those formed during the association.

Finally, we notice that the "static" force, namely the force calculated leaving the two subunits in their original native conformation, is remarkably different, stressing the role of protein relaxation in subunit-subunit interactions.

Protein Stability. To analyze the interactions at the subunit interface and compare them with those stabilizing the interior of the protein we calculate the distribution of the contacts [18] as a function of their energies at several subunit-subunit distances (Fig. 4). In the native state, the design procedure naturally favors stronger forces across the monomer-monomer interfacial contacts relative to the intra-chain ones. This is also in agreement with the suggestions coming from the experiments of Yip et al. [8]. As r increases the weakest inter-monomer bonds are broken and the strength distribution becomes more and more peaked around the strongest interaction. In contrast, the intra-chain bonds distribution remains almost unaltered as a results of the rupture of some contacts and formation of new ones.

Protein Deformation. Our calculations allow also for characterizing the deformation of the monomers as the dimer is pulled. The deformation is measured as the distance (by using the Kabsch expression [17]) of each monomer from the configuration it had in the native state, averaged over the two monomers (the values for the two monomers turn to be quite similar).

Fig. 5(a) shows the results of three simulations at temperature $T = 0.05\epsilon$ when the dimer is slowly pulled and then slowly released. It is visible that the final states are always the same but the pathways to reach them can be quite different. This is fully in agreement with the energy landscape theory and the protein folding funnel concept [19–25]. This behavior persists also at higher temperatures e.g. $T = 0.10\epsilon$. However, at more higher temperature after the dimer is released the native state is not more recovered and another locally stable configuration is reached ($T = 1.15\epsilon$ is shown in Fig. 5(b)). A similar situation occurs also at very low temperatures (Fig. 5(c)). After the dimer is pulled the system is unable to reach again its native state when it is released. This indicate that the process of pulling

and releasing is “reversible” only in a certain range of temperature. The temperature T has not to be too high (i.e. T has to be lower than a folding temperature T_f) otherwise the system escape from its local minimum and not too low (i.e. T has to be higher than a glassy temperature T_g) otherwise thermal fluctuations are not enough to allow the system to overcome the small scale roughness of the energy landscape (see for instance [22–24]).

Monomers’ refolding. In our ideal dimer, the primary structures for A and B are very similar but not equal. This is done to study the refolding process independently on the two monomeric subunits. Starting from swollen configurations well separated, the two monomers refold as two independent chains. We collected several configurations during MD simulations of these independent monomers. The minimum energy conformations of the couple of non-interacting monomers turns out to be composed by very similar A and B monomeric structures (0.7 Å of RMS difference) and not too different from the monomer structures constituting the dimer native state (about 2 – 3 Å each). The lowest energy conformations of the two non-interacting monomers are those reached at the final stage of the pulling process.

The similarity between the stable conformations of chains A and B folded separately could be predicted since their sequences are similar, although not identical.

The similarity between the independently folded conformations and those in the native protein agrees with the experimental results [6]. A conformational drift is experienced, as described before. Indeed, as Figure 6 illustrates, the refolded minimum-energy structure differs from the native one only for a rearrangement of the interfacial region.

Design quality. We have also addressed the question of how the width of the hysteresis cycle observed in fig. 3 depends on the design quality of sequences A and B . To further simplify matter we have restricted the number of amino acids classes to four, without loss of generality. The same structure of the dimer and the same design procedure [11] as above have been employed. However the design procedure was applied twice, one more and the other less accurate, providing two different pairs of sequences with the same native state and two different degrees of stability. The association and dissociation forces for these two

new cases are shown in Fig. 7 and clearly indicate the strong correlation between the design quality and the width of the hysteresis loop.

IV. DISCUSSION AND CONCLUSION

Our model of a dimeric protein, in spite of its extreme simplicity, is able to capture several specific features of the monomer/monomer interactions. Indeed, it reproduces the experimental "jump" behavior of the subunit-subunit force, which has been suggested to be a consequence of the disruption of a hierarchy of different kinds of intermolecular interactions (hydrogen bonds, electrostatic and dispersion forces and salt bridges) at the monomer–monomer interface. Furthermore, it provides hysteresis-like circles as observed in direct force experiments on dimeric proteins. Consistently, we find that the number of contacts between aminoacids at the interface is different in the association and in the formation processes. Finally, our calculations indicate that the native state of a dimeric protein is recovered if the two subunits are pushed back, again in agreement with experimental evidence [8]. The agreement with experiment is rather striking considering that it is achieved by designing the dimeric protein with a extremely simple force field (a Lennard–Jones potential) mimicking the complex interactions among amino acids. This strongly suggests that the characteristic feature of inter–monomer force curves can be simply a consequence of a design procedure optimizing the dimer structure with respect to myriad of possible alternative structures rather than to specific subunit-subunit interactions. This "design-based stabilization" is presently at the speculative level, yet it offers an explanation for the stability of specific dimers. For instance, the two monomers of insulin can form in principle two possible adducts basically energetically equivalent in terms of contact strength [1]. However, only one adduct is found in aqueous solution and in the crystal phase [2].

Furthermore, this work allows also for the first detailed theoretical characterization of the dynamics of association of a multimeric protein. First, it is shown that the design of the dimer is very highly optimized at the interface to stabilize the subunit-subunit interactions,

in agreement with the experimental observation that the quaternary structure of proteins is disrupted only in drastic conditions or by mutating key residues at the interface [6,2]. Second, we learn that at the first and most dramatic stages of the dissociation process, only the strongest interfacial interactions forces are maintained, whereas the intra-monomer interactions turn out to be almost unaltered. Consistently, we expect that the monomers do not rearrange significantly upon dissociation, the only region experiencing large rearrangements being at the interface. This is absolutely consistent with the recent high resolution structure of the monomeric form of the dimeric enzyme Cu,Zn superoxide dismutase [6], which exhibits minimal differences with the native protein.

The present results may be of help for protein-engineering experiment and for a deeper understanding of function/structure relationships of multimeric proteins.

V. ACKNOWLEDGMENTS

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A. Captions to the figures

Fig. 1. Native dimer: (a) Structure and numbering (indicated on each bead) of the type of the bead (as in column 1 of Table I); (b) Inner(squares)- and inter (dots)-chain contacts. In both figures, the chains A and B are represented in light and dark color, respectively. In our model, the sequences A and B are chosen not to be necessarily equal, in order to explore the relevance of the symmetry of the sequence for protein aggregation.

Fig. 2. Energy of the model protein plotted as a function of the distance d between subunits.

Fig. 3. Subunit dissociation (dark line) and re-association forces (light line) as resulting from constrained MD simulations where the two centers of mass of the two subunits are kept at fixed distance r . The forces are measured after equilibrium at temperature 0.06ϵ has been reached. Multiple points, very close to each other, indicate repeated calculations and give an estimate of the error bar for the various measured forces. The "static" force, indicated by the black line, is the force between the monomers immediately after they are separated out at distance r from their native state. The force vs r curve is then fitted with the function $F^{fit}(r) = \gamma\{(\frac{\rho}{r})^{\alpha_1} - (\frac{\rho}{r})^{\alpha_2}\}$ (best fit parameters $\gamma = 20.8\epsilon$, $\rho = 2.4\sigma_0$, $\alpha_1 = 22.8$ and $\alpha_2 = 8.5$, correlation coefficient among fitted and calculated values equal to 0.9998).

Fig. 4. Distributions of inter- (light shadow) and intra-monomeric interactions (dark shadow) normalized to the total number of contacts: at $r=15.5 \text{ \AA}$ (native state), (b) $r=23.4 \text{ \AA}$ and (c) $r= 30.6 \text{ \AA}$. Notice that stronger interactions are favored at the subunit-subunit interface with respect of the intramonomer interactions.

Fig. 5. Monomer deformation (distance of a monomer from the corresponding native structure, averaged over the two monomers) as a function of the distance r at $T= 0.05 \epsilon$ (a), $T=0.15 \epsilon$ (b) and $T=0.01 \epsilon$ (c).

Fig. 6. Comparison between the optimized structures of one subunit folded as in the native protein (dark line) and as independent monomer (light line). Residues at the interface are indicated with circles.

Fig. 7. Dissociation and re-association forces for two dimers with the same native state but different pairs of sequences: one obtained with an optimal design (*a*) and the other with a poor design quality (*b*). Hysteresis loop width correlates well with the degree of dimer stability.

TABLES

TABLE I. Number of amino acids of each kind, occurring in A and B sequences (column 3), according to their occurrence in proteins (column 2).

Residue	Occurrence	Occurrence
Type	in Proteins (%)	in our model (#)
(1)	(2)	(3)
CYS (type 1)	1.7	1
MET (type 2)	2.4	2
PHE (type 3)	4.1	3
ILE (type 4)	5.8	3
LEU (type 5)	9.4	5
VAL (type 6)	6.6	4
TRP (type 7)	1.2	1
TYR (type 8)	3.2	2
ALA (type 9)	7.6	4
GLY (type 10)	6.8	4
THR (type 11)	5.7	3
SER (type 12)	7.1	3
ASN (type 13)	4.5	2
GLN (type 14)	4.0	1
ASP (type 15)	5.3	2
GLU (type 16)	6.3	3
HIS (type 17)	2.2	1
ARG (type 18)	5.1	2
LYS (type 19)	6.0	2
PRO (type 20)	5.0	2

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FIGURES

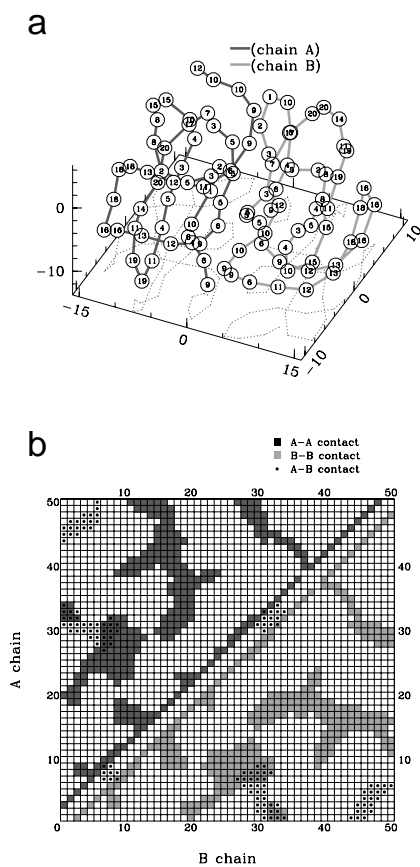


FIG. 1.

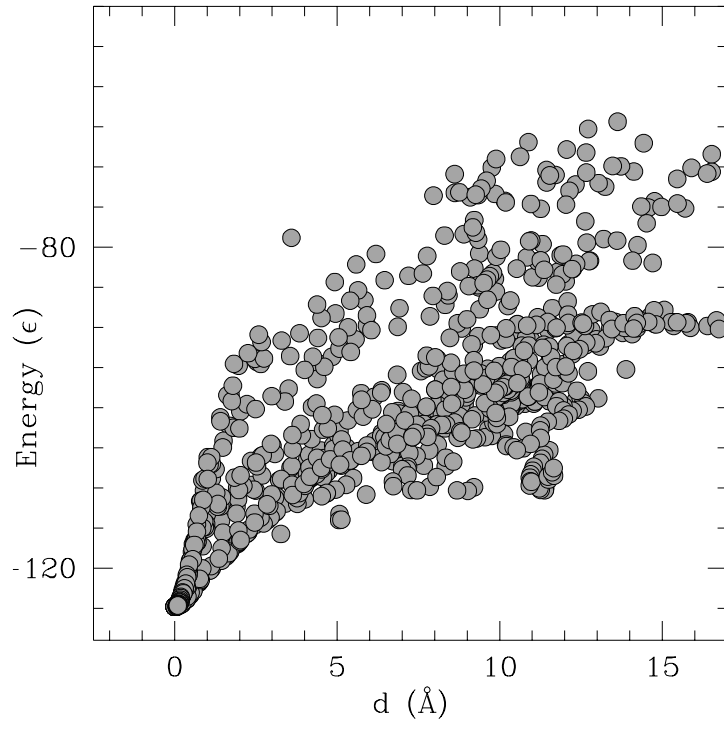


FIG. 2.

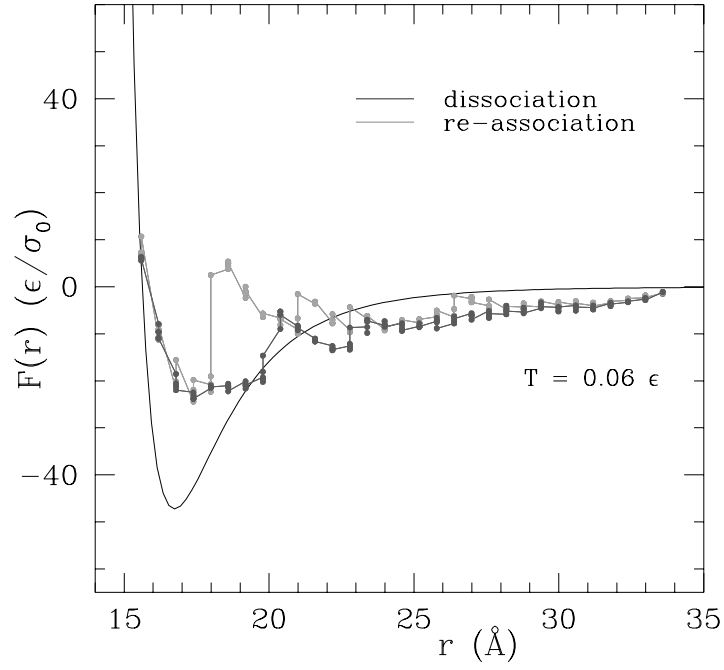


FIG. 3.

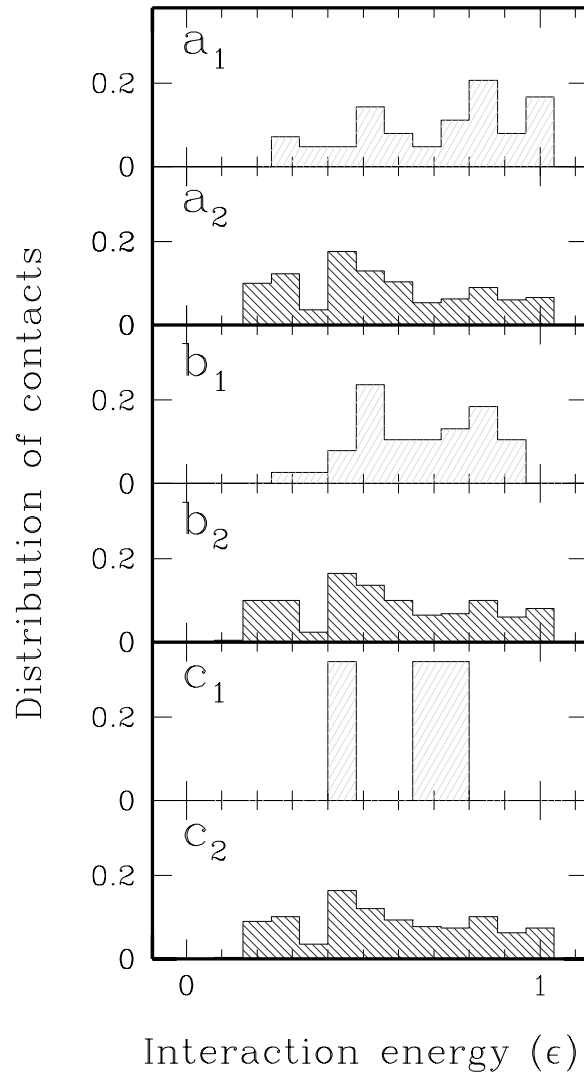


FIG. 4.

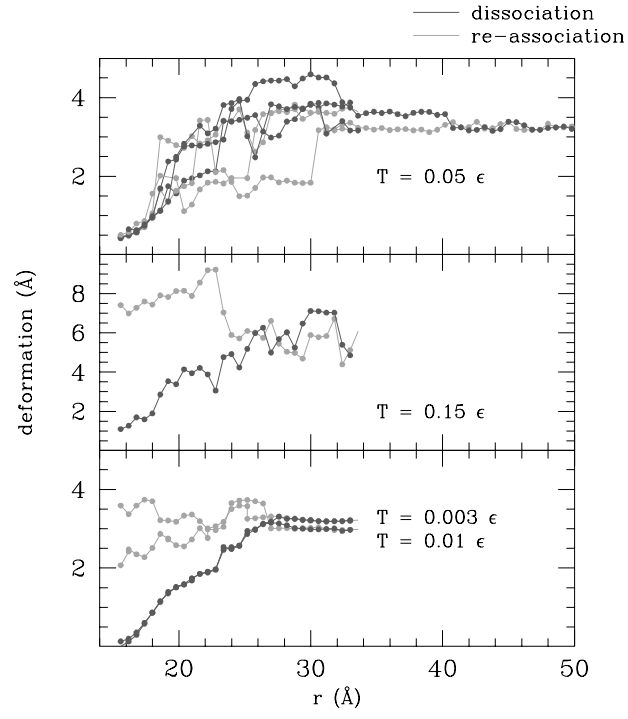


FIG. 5.

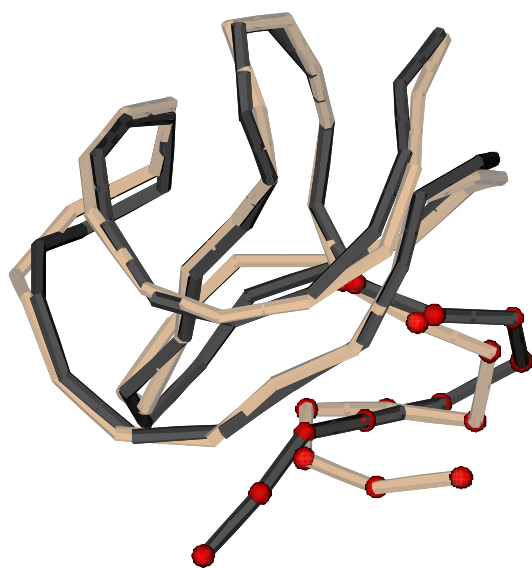


FIG. 6.

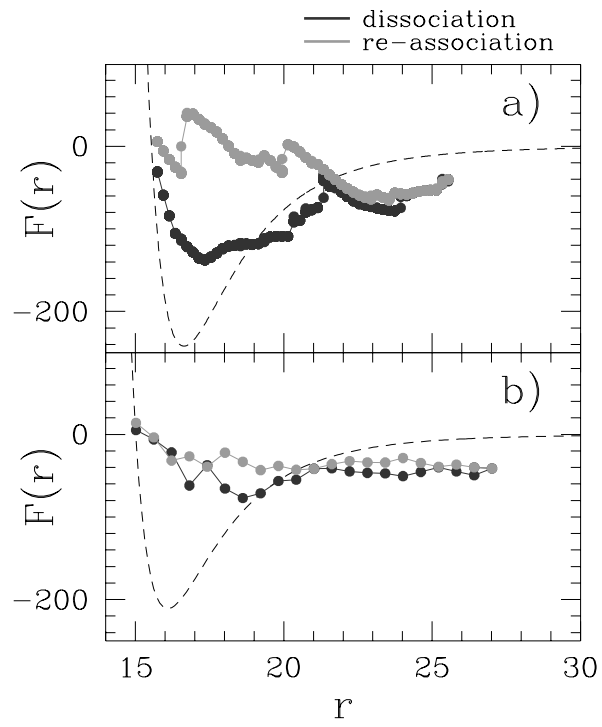


FIG. 7.